

MORPHOLOGICAL DIFFERENCES BETWEEN *OPHIOSTOMA PICEAE* AND *O. QUERCI*, AND AMONG *O. QUERCI* ISOLATES

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ABSTRACT - *Ophiostoma querci* differs statistically from *O. piceae* in lengths of synnematal conidiogenous cells, perithecial neck, ostiolar hyphae. Also the differences in synnemata height, ascocarp base diameter, ascospores shape and in the germination rate of synnematal conidia on SNA at 25°C were found. Differences in colony morphology, synnematal production and length of the *Sporothrix* primary conidia among *O. querci* isolates are also presented. The atypical strain 88A from Poland (only in anamorphic condition but compatible with the mating type A of *O. querci*) could be a new variety of this species.

RÉSUMÉ - Cette étude montre qu'il est possible de distinguer par des critères morphologiques les deux espèces jumelles *Ophiostoma querci* et *Ophiostoma piceae*. Elle a porté sur 14 souches monospores d'origines variées (10 *O. querci* dont les 2 géniteurs A et B du néotype de *O. querci*, et 4 *O. piceae*). L'incompatibilité sexuelle de ces 2 espèces, la première inféodée aux Feuillus, la seconde aux Conifères a été vérifiée. Puis des différences significatives ont été relevées aux niveaux de la longueur des cellules conidiogènes des corémies (plus longues chez *O. querci*); - de la longueur du col des périthèces (plus long chez *O. querci*); - de la longueur des soies ostiolaires (plus longues chez *O. piceae*). Des différences existent aussi en ce qui concerne la hauteur des corémies, le type de germination des conidies de ces dernières sur milieu SNA à 25°C, le diamètre des ascocarpes et la forme des ascospores. L'étude comparative des anamorphes de *O. querci* révèle une bonne homogénéité ■ niveau des caractères culturels, de la production des corémies et de la taille des ramoconidies du stade *Sporothrix*, excepté pour la souche 88A d'origine polonaise. Celle-ci appartient à l'espèce *O. querci* puisque son croisement avec le géniteur A de *O. querci* est fertile, mais pourrait constituer une variété morphologique de cette espèce, du fait de ses caractéristiques qui la rapprochent du *Hyalodendron roboris* de Georgescu et al.

KEY WORDS : *Ophiostoma piceae*, *Ophiostoma querci*, *Quercus* spp., Oak, *in vitro* study.

I. INTRODUCTION

Ophiostoma piceae (Münch) H. et P. Sydow has been considered for a long time (Hunt, 1956, sub. nom. *Ceratocystis piceae* (Münch) Bakshi) as a fungus inhabitant of both hardwoods and conifers. Among its synonyms is *Ophiostoma querci* (Georg.) Nannf. (sub. nom. *Ceratostomella querci* Georgevitch) described from oak. This point of view was shared till recently by Upadhyay (1981) and Przybyl & de Hoog (1989).

Separation of these two taxa was proposed by Brasier & Kirk (1989) and Brasier & Webber (1990). The first mentioned of the above authors discovered that *O. piceae* isolates from hardwoods and conifers are reproductively incompatible, and although "morphologically similar, may be sibling species of *Ophiostoma*". The former authors concluded: "the name *O. querci* may need to be re-established for the hardwood taxon".

As no authentic material was available for *O. querci*, Morelet (1992) proposed for it a neotype from oak, allowing the application of this name to hardwood taxon. He also suggested ■ complementary morphological study between *O. piceae* and *O. querci*.

Furthermore, ■ strain of *Sporothrix* (88A) was isolated from a *Quercus robur* trunk in the Krotoszyn Forest District in Poland (Przybyl, in print), similar in the morphology of primary conidia to those of *Hyalodendron roboris* Georgescu and Teodoru (anamorph of *O. roboris*).

The aim of our work was to find morphological differences between *O. piceae* and *O. querci* and also to identify the taxonomic position of 88A strain.

II. MATERIAL AND METHODS

The 14 strains selected for experiments are listed in Table 1, and the following studies were carried out:

Colonies characters

The fungi were grown in Petri dishes with MA 3% (Malt agar Difco, pH 5.6) at 25°C in darkness. The radial growth of mycelium and colony characters of all studied strains were noted after 10 days, in 6 replications.

Anamorph characters

1. Synnemata

For examination of synnematal conidiogenous cells the isolates were grown in Petri dishes with BWA (Beer Wort Agar) at 25°C in darkness and examined after 10-15 days;

For measurements of synnemata height and of conidia length from synnemata, the isolates were grown in Petri dishes with MA at 25°C and synnemata were sampled after 10-15 days.

For the purpose of observation of their germination rate, drops of synnematos conidia suspension of *O. querci* and *O. piceae* were transferred separately onto SNA medium (Nirenberg, 1976) and incubated at 25°C in darkness.

2. *Sporothrix* primary conidia

The isolates were grown in Petri dishes with MA at 25°C in darkness and studied over 10-15 days. For the measurement of primary conidia the scant mycelium was taken when two colony types (scant and floccose) occurred.

Cultures	Locations	Fungi	Hosts
O.2	Cerilly, France	<i>O. quercus</i> mating type A of neotype	<i>Quercus petraea</i>
O.80	Nogent sur Vernisson, France	<i>O. quercus</i> mating type ■ of neotype	<i>Quercus</i> sp.
O.91	Blois, France	<i>O. quercus</i>	<i>Q. petraea</i>
10A5	Krotoszyn, Poland	<i>O. quercus</i>	<i>Q. robur</i>
88A	Krotoszyn, Poland	<i>O. quercus</i>	<i>Q. robur</i>
100.69 CBS	Northern Ireland	<i>O. piceae</i>	<i>Picea sitchensis</i>
195A	Krotoszyn, Poland	<i>O. quercus</i>	<i>Q. robur</i>
244A	Sulechów, Poland	<i>O. quercus</i>	<i>Q. robur</i>
276	Sulechów, Poland	<i>O. quercus</i>	<i>Q. robur</i>
318	Tuliszów, Poland	<i>O. quercus</i>	<i>Q. rubra</i>
336	Myslibórz, Poland	<i>O. quercus</i>	<i>Q. robur</i>
468	Czechoslovakia	<i>O. piceae</i>	<i>Pinus sylvestris</i>
469	Czechoslovakia	<i>O. piceae</i>	<i>Picea abies</i>
470	Czechoslovakia	<i>O. piceae</i>	<i>Picea abies</i>

Table 1. Details of *Ophiostoma quercus* and *O. piceae* studied.

Strains from Czechoslovakia and from CBS (the Netherlands) were sent to K. Przybyl by J. Franknechtova in year 1991 and S. de Hoog in year 1988 respectively. The other strains were isolated and recognized by the authors in own laboratories.

Les isolats de Tchécoslovaquie et du C.B.S. ont été envoyés respectivement en 1991 par J. Franknechtova et en 1988 par S. de Hoog. Les autres ont été isolés et identifiés par les auteurs.

3. Measurements

Fifty measurements of the length of both the synnematal conidiogenous cells and *Sporothrix* primary conidia were made for each isolate growing in the conditions mentioned above. Pairwise comparisons of length means of synnematal conidiogenous cells were carried out with the LSD analysis of variance and in the case of *Sporothrix* primary conidia the Newman-Keuls method was used.

Teleomorph characters

Neck and ostiolar hyphae were examined from the perithecia produced on oak sapwood in using general method of mating reactions (Morelet, 1992).

1. Mating reactions

Crosses (with 3 replications of each) were carried out in the following combinations:

- between types A x B of *O. quercu* isolates,
- between types A x B of *O. piceae* isolates
- between 88A and *O. quercu* isolates,
- between 88A and *O. piceae* isolates.

2. Ascocarps

For examination 10 perithecia were taken from each replication (30 for each combination) of *O. quercu* and *O. piceae* isolates 15 days after pairing. Lengths of perithecial neck and ostiolar hyphae were measured and statistically analysed with the Newman-Keuls method.

Also the drop of ascospores, collected from perithecia formed as a result of positive mating reaction, between 88A and *O. quercu* isolate, was transferred onto MA (pH 5.6) and incubated for the first 10 days at 25°C in darkness and then at room temperature in light. Moreover, single - ascospore cultures were made using strongly diluted spore suspensions and taking each ascospore under lens microscope.

III. RESULTS

Colony morphology

Colonies attained over 10 days a diameter of 55-67 mm (an average of 64 mm) for *O. quercu*, of 67-78 mm (an average of 72 mm) for *O. piceae*. Aerial mycelium was scant - floccose usually growing in sectors, whitish grey for *O. quercu* isolates, felted white for 88A, felted, grey for *O. piceae*. Reverses were pale brownish in *O. quercu* and *O. piceae* but uncoloured in 88A. Synnemata were produced abundantly all over the colonies or in the concentric zones of *O. quercu*, frequently or abundantly over the colonies or in a broad band near the colony of *O. piceae*, whereas they were absent in the colony of 88A. Significant is the fact, that the synnemata in 88A cultures did not arise during 2 months of observations on MA at 25°C but were easily found on sapwood samples 2 weeks after inoculation.

Anamorphs characters

1. Synnemata

Lengths of branched and unbranched synnemata were between (130-) 350-500 (-600) µm for *O. quercu* whereas for *O. piceae* between 200-700 (-800) µm.

Lengths of conidiogenous cells of synnemata ranged between 8.3 - 16.6 μm for all isolates of *O. quercii* and 8.3 - 13.4 μm for isolates of *O. piceae*. Length means, of *O. quercii* and *O. piceae* ranged for all studied isolates between 13.3 - 14.2 μm and 10.1 - 10.9 μm respectively. The values of length means of *O. quercii* and *O. piceae* isolates were significantly different with a confidence level of $\alpha = 0.05$ in LSD analysis (Table 2).

In regard to the germination of synnematal conidia, various sizes of conidia resembling yeast-form cells, oblong to globose both in *O. quercii* and *O. piceae* occurred on SNA at 25°C, after 24 hours. Differences between them in the germination rate of conidia were observed in 3 days. In *O. quercii* well developed germinated tubes were found, whereas in *O. piceae* only initial germinations of conidia were observed.

FUNGI	STRAINS	MEANS ^a
<i>O. quercii</i>	10A5	14,28 a
	195A	14,08 ab
	336	14,01 ab
	318	13,81 ab
	244A	13,74 ab
	88A**	13,74 ab
	276	13,28 b
<i>O. piceae</i>	468	10,86 c
	469	10,46 c
	180.69	10,13 c

Table 2. Pairwise comparisons of length means (μm) of synnemata conidiogenous cells.

Tableau 2. Comparaison de moyennes deux à deux des cellules conidiogènes du stade *Graphium* (longueur en μm).

^a The data with common letters do not differ significantly ($\alpha = 0.05$) with using the LSD method. Les données affectées d'une même lettre ne diffèrent pas significativement au seuil de 5% (test L.S.D.).

** 88A - The measurements of synnemata conidiogenous cells on sapwood samples.

Les synnema utilisés pour les mesures des cellules conidiogènes ont été prélevés sur milieu gélosé sauf dans le cas de 88A où ils l'ont été sur aubier de chêne.

Isolates	MA 25°C		
	Range	Mean value*	
88A	6,64 - 39,64	25,56 a	O
			P
O.2	8,30 - 18,26	13,28 b	H
			I
O.80	8,30 - 23,24	12,55 b	O
			S
10A5	8,30 - 19,92	12,82 b	T
			O
195A	6,64 - 23,24	14,24 b	M
			A
244A	6,64 - 26,56	12,82 b	
			Q
276	8,30 - 26,56	15,39 b	U
			E
318	6,64 - 23,24	13,76 b	R
			C
336	8,30 - 26,56	13,96 b	I
180.69	6,64 - 21,58	14,10 b	O.
			P
468	9,96 - 28,22	15,77 b	I
			C
			E
469	9,96 - 28,22	14,04 b	A
			E

Table 3. Length of primary conidia (μm).Tableau 3. Longueur des ramoconidies (μm).

*Data with common letters do not differ significantly ($\alpha = 0.01$) with using Newman-Keuls method.

Les données affectées d'une même lettre ne diffèrent pas significativement au seuil de 1% (Test de Newman-Keuls).

2. *Sporothrix* primary conidia

Primary conidia formed by sympodial growth on the apical part of the conidiogenous cells were hyaline, thin - and smooth walled, clavate, 0-2 septate for all studied isolates of *O. quercii* (except 88A with 4% of 3-septate primary conidia) and 0-2 for *O. piceae*. Length of primary conidia ranged from 6.6 - 26.6 μm for the 8 *O. quercii* isolates, (6.6-39.6 μm for 88A) and 6.6 - 28.2 μm for *O. piceae*. Maximal means length among all studied isolates reached 15.4 μm for *O. quercii* and 15.8 μm for *O. piceae*. Isolate 88A with maximal mean length 25.6 μm differed statistically from *O. quercii* and *O. piceae* values with confidence level of $\alpha = 0.01$ in the Newman-Keuls method (Table 3).

Teleomorph characters

1. Mating reactions

Positive mating reactions were observed only:

- between compatibility types of *O. quercii* isolates
- between compatibility types of *O. quercii* isolates and 88A
- between compatibility types of *O. piceae* isolates.

2. Ascocarps

Ascocarp bases for *O. quercii* and *O. piceae* were between 90-160 (-190) μm and 100-180 (-200) μm respectively.

O. quercii and *O. piceae* differed considerably in their length averages of both neck and ostiolar hyphae:

The mean length of *O. quercii* neck, 1515 μm (maximally 1900 μm), was statistically larger than for *O. piceae*, where the average value amounted to 1092 μm and maximal value to 1600 μm . On the other hand the ostiolar hyphae length of *O. quercii*, about 15 in number, was significantly smaller than for *O. piceae* about 25 in number. In the case of *O. quercii* the ostiolar hyphae length was noted up to 36 μm (average value 21 μm) whereas in *O. piceae* it was up to 55 μm (average number 38 μm - Table 4).

Ascospores ranged (3.5-) 2.5 x 1.5 μm both for *O. quercii* (allantoid in side view) and *O. piceae* (reniform in side view).

Ascospores from perithecia which were formed in cross 88A x *O. quercii* were fertile. Indeed perithecia of F2 filial generation were obtained.

IV. CONCLUSIONS

Differences between *O. quercii* and *O. piceae*

O. quercii differs statistically from *O. piceae* in lengths of synnematal conidiogenous cells (longer in *O. quercii*), perithecium neck (longer in *O. quercii*) and ostiolar hyphae (longer in *O. piceae*).

Differences between 88A and *O. quercii* isolates.

Isolate 88A differs from *O. quercii* isolates in colony morphology (scant, floccose for 8 isolates, against felty for 88A), synnemata formation (they were absent during 2 months of observation in 88A) and length of *Sporothrix* primary conidia. In the case of 88A the length of primary conidia ranged between 6.64-39.64 μm (mean value

Mating reactions	Neck length (μm)	Ostiolar hyphae length (μm)
<i>O. querci</i>		
0.2 x 276	1200 - 1900	16,60 - 26,56
0.2 x 0.91	1200 - 1900	16,60 - 33,20
0.2 x 0.80	1100 - 1800	16,60 - 36,52
0.2 x 88 A	1200 - 2500	11,62 - 19,92
<i>O. piceae</i>		
180,69 x 468	900 - 1400	26,56 - 54,78
180,69 x 469	800 - 1600	21,58 - 49,80
180,69 x 470	600 - 1500	16,60 - 53,12
Average		
<i>O. querci</i>	1515,45 ■	21,31 a
<i>O. piceae</i>	1091,65 b	38,14 b

Table 4. Results of mating reactions.

Tableau 4. Longueur des cols et hyphes ostiolaires des périthèces obtenus en croisements *in vitro*.
 Average number differ significantly ($\alpha = 0.05$) with using Newman-Keuls method.
 Les moyennes diffèrent significativement au seuil de 5% (Test de Newman-Keuls).

25.56) and therefore was nearby those of *Hyalodendron roboris* 3.5-45 μm (Georgescu et al., 1948). Noted by mentioned authors 3-septate primary conidia were also observed in 88A isolate. The 88A gave fertile perithecia (with longer neck and shorter ostiolar hyphae, see Table 4) in cross with O.2 (the mating type A of the neotype of *O. querci*).

Descriptions of *O. querci* (including its neotype) and *O. piceae* based on our material

Ophiostoma querci (Georgevitch) Nannfeldt.

Synanamorphs: *Graphium pirinum* Goidanich

Sporothrix pirinum (Goidanich) Morelet

Colonies (55-) 57-67 mm in diam. over 10 days on MA at 25°C; aerial mycelium scant and floccose, whitish grey; growing in sectors; reverses pale brownish. Synnematal unbranched and branched (130-) 350-500 (-600) μm height, all over the colony or in the concentric zones. Synnematal conidiogenous cells 8-16 μm long produce slimy masses of oblong conidia (4.5-) 2.5 x 1.5 (-2.5) μm . *Sporothrix* primary conidia 7-27 μm long, hyaline, thin - and smooth walled, clavate, 0-2 septate.

Ascarps partly embedded in the agar medium; bases brown, globose 90-160 (-190) μm in diam.; necks dark brown at the base, lighter toward the apex, 1100-1900 μm long including ostiolar hyphae; ostiolar hyphae about 15 in number, brownish at the base, often septate up 37 μm ; ascospores allantoid in side view (3.5-) 2.5 x 1.5 μm .

The atypical morph 88A (only in anamorphic condition) differs from the above typical description in having aerial mycelium felty white, reverse uncoloured, synnemata production very slow after 2 months of incubation. *Sporothrix* primary conidia 0-3 septate up 40 μm long. Then 88A could be considered ■ a new variety into *O. querci*.

Ophiostoma piceae (Münch) H. & P. Sydow.

Synanamorphs: *Graphium piceae* (Crane & Schoknecht) Wingfield & Kendrick
Sporothrix sp.

Colonies attained a diameter of 79 mm (67-79 mm) over 10 days on MA at 25°C; aerial mycelium grey and felty, with frequent or abundant synnemata arising over colonies or in the broad band; reverses at first white later becoming pale brownish. Synnemata unbranched and branched 200-700 (-800) μm height; conidiogenous cells 8-13 μm long with slimy masses of oblong conidia (4.5-) 2.5 x 1 (-1.5) μm . *Sporothrix* primary conidia 7-28 μm long, hyaline, thin and smooth walled, clavate, 0-2 septate.

Ascarps partly embedded in the agar medium; bases globose (sometimes elliptical) dark 100-180 (-220) μm in diam.; necks black at the base, becoming lighter at the apex, 600-1600 μm long including ostiolar hyphae. Ostiolar hyphae about 25 in number, septate up 55 μm long; ascospores reniform in side view (3.5-) 2.5 x 1.5 μm .

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